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SOME SUGGESTIONS CONCERNING THE BACTERIOLOGICAL DIAGNOSIS OF HUMAN BOTULISM.

By K. F. MEYER, of the George Williams Hooper Foundation for Medical Research, University of California, and J. C. GEIGER, Epidemiologist, United States Public Health Service.

Considerations Prompting Bacteriological Study.

In the course of an analysis of a fairly large number of outbreaks of botulism in man reported during the last 10 years, it was noted that the diagnosis was frequently based on clinical symptoms only. Sometimes it was possible to demonstrate the botulism toxin or the organisms in the causative food. In numerous instances, however, the remnants of the suspected food were evidently not available or were examined by a chemist instead of a bacteriologist. From a medico-legal, and also from an epidemiological, standpoint, the diagnosis could therefore be questioned, and the statistician attempting to unravel the all-embracing diagnosis "ptomaine or food poisoning" finds little definite information in the published reports. If one recalls, moreover, that botulism can be mistaken by the inexperienced person for methyl-alcohol poisoning, encephalitis lethargica, or cerebrospinal syphilis, it is obviously important to conduct careful necropsies on such cases and to determine either by cultural or histological studies the true nature of the disease. In connection with the latter procedure it must be said that the characteristic thrombi or prethrombotic stages in the arteries and veins of the meninges and brain originally described by Ophüls (1) may not be present, and a microscopic study alone may therefore fail to render a diagnosis. Bacteriologic studies of the tissues of fatal cases have been made in a few instances. V. Ermengem (2), Ornstein (3), and Graham (4) report the demonstration of *B. botulinus* in the spleen of fatal botulism cases. Some writers also recommend a cultural study of the intestinal contents at autopsy, but nothing is said regarding the possibility of finding *B. botulinus* in the stools of clinical cases.

Theoretically, at least, stool examinations appear to be valuable, inasmuch as numerous observers have found this organism in the excreta of animals which ingested food spontaneously or experimentally contaminated with the poison and spores of the organism. Constipation is almost a constant manifestation of botulism and is naturally conducive to the persistence of the organism for a considerable period in the alimentary canal of man and animals. This must be particularly the case in those instances in which a bowel

movement can not be procured, in spite of all medication, until the 10th or even the 16th day, as reported by Schumacher (5).[1]

These and other considerations to be discussed elsewhere prompted us to study bacteriologically some cases of botulism which came to our notice during the last 12 months. The findings thus far made are suggestive and are reported in order that other workers may amplify our observations when the occasion arises.

Methods Employed in Culturing Tissues or Stool Specimens for B. botulinus.

Portions of the organs are ground in a mortar with sea sand and emulsified with saline. Stool specimens are diluted with saline until the formed portions are finely divided. The emulsions are placed in 250-c. c. culture flasks and heated for one hour at 60° C. They are then mixed with 100 c. c. sterile beef heart medium, which consists of one part of ground beef heart and two parts of peptic digest broth of a reaction P_H 7.4. The mixture is stratified with oil, or, better, with vaseline. The flasks, which are closed with rubber stoppers and sealed with Imperial or Major's glue, are exhausted of air as completely as possible. After incubation for from 10 to 30 days at 37° C., the centrifugalized supernatant fluid is administered, in 1- to 2-c. c. doses, to guinea pigs. The presence of *B. botulinus* toxin is definitely ascertained by a neutralization test with known type A, and B, *B. botulinus* antitoxic sera. Isolation of *B. botulinus* is accomplished by fractional heating, enrichment, and deep cultivation in liver-peptone agar. Heating of the emulsions at 60° C. for one to two hours alone insures the possibility of obtaining proper cultures, as is shown in the case reports.

Report of Cases.

Richmond, Calif., outbreak (Feb. 25, 1920).—*B. botulinus* type A, and *B. botulinus* type A toxin were demonstrated in a can of olive relish, responsible for one fatal case. Anaerobic cultures from the spleen of the patient (25 grams), liver (20 grams), lung (10 grams), kidneys (9 grams), mucus from ileum (5 grams), and jejunum were negative for *B. botulinus*. The intestinal wall was not cultured.

Florence, Ariz., outbreak (May, 1920).—Canned beets were suspected as the causative food. For a bacteriological examination, the spleen (weight 242 grams), a portion of the jejunum, and the brain of Ch. R., who died on May 19, 1920, were available.

Cultures of the spleen (30 grams) and chyme (5 c. c.) of the intestinal loop demonstrated *B. welchii*, *B. sporogenes*, and *B. bifermentans*.

[1] References:

(1) Arch. Int. Med. 1914, 14, p. 589.
(2) Ztschr. f. Hyg. u. Infektionskr. 1897, *26*, p. 4.
(3) Ztschr. f. Chemotherap. Orig., 1913, *1*, p. 458.
(4) McCaskey: Am. Jour. Med. Sc. 1919, *158*, p. 57.
(5) Münch. Med. Wchnschr. 1913, *60*, p. 124.

A specimen consisting of 4 grams of macerated jejunal wall gave a culture of *B. botulinus*, type B, associated with *B. tertius*, *B. welchii*, *B. sporogenes*, *B. tetano morphus*, and two other unidentified anaerobes. The strain of *B. botulinus* was isolated in pure culture. The remaining portion of the intestinal wall, which had been kept in the ice chest for four weeks, had undergone autolysis and decomposition, but when cultured, *B. botulinus*, type B, was isolated. Sections of the brain revealed definite prethrombotic stages in the blood vessels of the brain. Cultures of the brain revealed cocci and gram negative aerobic rods.

Oakland, Calif., outbreak (October, 1920).—Canned spinach was suspected as the causative food. Available for a bacteriological study were some stool specimens collected from Miss A. R., who recovered from the disease. The responsible meal was consumed on October 14, 1920; the first symptoms were noted on the 16th; intestinal washings were obtained on October 20, and a formed stool on October 21, 1920. Six specimens of 75 c. c. each of the intestinal washings were heated for one hour at 60° C. and cultured; five contained *B. botulinus*, type A. Five specimens of 75 c. c. each were cultured in an unheated state. These cultures, on repeated tests, were negative for *B. botulinus*. Three samples of 50 grams of solid stool were emulsified in saline, heated at 60° C. for one hour and cultured. Only one sample gave a culture of *B. botulinus*. Three unheated specimens of the same sample were negative. *B. botulinus* was, therefore, present in six stool specimens collected on the sixth and seventh days, respectively, after the consumption of the causative meal.

Grand Rapids, Mich., outbreak (January, 1921).—Canned spinach was suspected as the causative food. Through the courtesy of Dr. Merrill Wells the intestinal washing (enema) of Miss H. was collected on the eleventh day and made available on the seventeenth day after the consumption of the infected meal. One flask out of five stool specimens of 50 c. c. each, which had been heated to 60° C. and cultured, contained, on the tenth day of incubation, *B. botulinus* toxin, type A. The isolation of the organism in pure culture is in progress. Two unheated specimens were negative. *B. botulinus* was, therefore, present in a stool specimen on the eleventh day after the causative meal had been consumed.

Comment.

The foregoing observations indicate that the spores of *B. botulinus*, when presumably ingested in the poisonous food, may remain in the intestinal canal and may be eliminated in the stools of typical botulism cases. Several important problems suggest themselves for immediate experimental study or investigations on human cases of

this disease; the following points deserve particular consideration: (1) Determination of the average period of fecal discharge of *B. botulinus* spores in severe and mild cases of botulism; (2) quantitative estimation of the eliminated spores per 1 or 10 grams of enema or formed stool; (3) quantitative comparison of the spore content of the causative food and that of the stools; (4) testing of filtered stool suspensions, on guinea pigs, for the presence of toxin; and (5) testing for *B. botulinus* spores the stools of normal human beings who eat raw fruit or vegetables and live in districts in which the organism is quite common in the soil.

These investigations would undoubtedly contribute information as to the possible pathogenicity of *B. botulinus* spores as suggested by Orr (6), Edmondson, Giltner, and Thom (7), and others. *B. botulinus* possesses a noteworthy degree of growth adaptability, and it is possible that the spores can germinate in the paretic intestinal tube and form toxin. Some personal observations on spontaneously diseased domesticated animals justify this suggestion. It appears also of importance to know if botulism convalescents can remain "spore carriers" and, as such, assist in the progressive pollution of the earth with dangerous bacteria. The diagnostic value of the demonstration of *B. botulinus* spores can only be accepted when repeated tests on normal stool samples have demonstrated an absence of this organism. An experimental study of the problem mentioned under (5) in the preceding paragraph is in progress. The examination of numerous sewage samples of urban and rural origin has thus far failed to give positive *B. botulinus* cultures, and we therefore feel confident that the stool test will be of practical value. However, it remains to be demonstrated as to the number of days a botulism patient is capable of discharging *B. botulinus* spores. The observations made by Thom, Edmondson, and Giltner (8), and others on guinea pigs strengthen our belief that the spores may be demonstrated only in the fecal remnants of the causative meal. Inasmuch as the discharge of this material is quite often delayed for many days, on account of the intestinal paresis, positive findings may be recorded for two, perhaps even three, weeks.

A quantitative estimation of the spores in the stool samples or in the causative food offers no technical difficulties. For example, in one of the recent outbreaks the spinach responsible contained *B. botulinus* spores in practically a pure state. Shake cultures and particularly those in dried liver agar gave colonies which could be readily counted.

The finding of *B. botulinus* spores in the jejunal wall, but not in the chyme, of the particular intestinal loop mentioned may be merely accidental or may vaguely support the recently advanced, but rather fanciful, conception (9) that "*B. botulinus* when taken into the human

system lodges in the digestive tract, and the toxins produced there spread over the body." It is our intention to discuss this phase of botulism elsewhere in detail; nevertheless, the diagnostic significance should be emphasized. We had recently occasion to study, in cooperation with Dr. L. R. Vawter, of Reno, Nev., a cattle disease in which *B. botulinus* apparently exhibited invasive properties. Invariably the organism was isolated from the inflamed duodenum and jejunum, the liver, mesenteric lymph-nodes, etc.

It is noteworthy that our two attempts to isolate *B. botulinus* from the spleen were not successful. These results may, in part, be due to the fact that post-mortem invasion was made impossible by the early removal and careful preservation of the tissues after death.[1]

Summary.

B. botulinus, type B, has been isolated from the jejunal wall of a case of botulism fatal on the fifth day of the disease. Spleen cultures in two instances were negative for *B. botulinus.* Stool specimens of two clinical cases of botulism, obtained from two different outbreaks, contained *B. botulinus,* type A, on the sixth, seventh, and eleventh day, repectively, after the consumption of the causative meal. The methods of tissue and stool cultures are described. The importance of culturing the stools and tissues of all clinical cases of botulism is evident.

THE COMPARATIVE TOXICITY OF THYMOL AND CARVACROL (ISOTHYMOL.)[2]

By A. E. Livingston, Physiologist, United States Public Health Service.

Introduction.

Uncinariasis was shown by Stiles in 1903 to be quite prevalent in the southern portion of the United States, and his efforts are largely responsible for the fact that it is commonly diagnosed as such in this country at the present time. The treatment, which is now recognized as an important economic problem in many localities, usually consists of some vermifuge which will either kill or paralyze the parasite, causing it to release its hold on the intestinal wall and thus be swept from the digestive tract with the excreta. The ideal treatment should quickly kill all the parasites and at the same time produce no undesirable effects on the patient. Such a substance has not thus far been found. Among the various remedies which have been used may be mentioned

[1] References:
(6) Proc. Soc. Exp. Biol. and Medicine, 1919, 17, p. 47.
(7) Arch. Int. Med., 1920, 26, p. 357.
(8) Jour. Am. Med. Assn., 1919, 73, p. 911.
(9) Boston Med. & Surg. Jour., 1920, July 29, 183, p. 139.

[2] From the Division of Pharmacology, Hygienic Laboratory, United States Public Health Service.